United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	Use of echocardiography and isolated perfused organ preparations to investigate cardiovascular impacts of air pollution inhalation in mice
LAPR Number:	17-05-001
Principal Investigator	Exemption 6
Author of this Document:	Exemption 6/RTP/USEPA/US
Date Originated:	04/10/2014
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Agenda Date:	04/23/2014
Date Approved:	05/19/2014
Date Closed:	05/05/2017

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6/RTP/USEPA/US by Exemption 6 RTP/USEPA/US	05/19/2014	Designated Reviewer	
	Exemption 6/RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	05/19/2014	DMR	

Administrative Information

1. Project Title (no abbreviations, include species):
Use of echocardiography and isolated perfused organ preparations to investigate cardiovascular impacts of air pollution inhalation in mice

Is this a continuing study with a previously approved LAPR?
No

2. What is the Intramural Research Protocol (IRP) number covering this project?
IRP-NHEERL-RTP/EPHD/CIB/Exemption Exemption Exemption/14-01-001.

These studies will provide research findings that directly address the following explicitly stated needs of the United State Environmental Protection Agency's (US EPA) Office of Air and Radiation: 1) Increased understanding of true multipollutant effects/impacts (single pollutants acting in combination) and 2) disentangling single pollutant effects/impacts in multipollutant exposures. This research is part of the US EPA's Air Climate and Energy (ACE) Research Action Plan (ACE Task 045). In addition, the elucidation of the mechanisms that drive adverse cardiovascular effects of air pollution is necessary to reduce uncertainty in standard setting and facilitate identification of hazard potential and assessment of risk to human health.

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	Exemption 6 ***Lotus Notes Address*** Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US	EPHD ***Branch*** CIB	MD

4. Alternate Contact:

Alternate Contact	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	919-541-4767 ***Lotus Notes Address*** Exemption 6 Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US	EPHD ***Branch***	MD

SECTION A - Description of Project

1. Study objectives, presented in <u>non-technical language</u> such that it is understandable by non-scientific persons, including how the study addresses health protection. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.

Air pollution inhalation increases cardiovascular morbidity and mortality. Such effects often result from localized, short term spikes in air pollution (comprised of complex mixtures of multiple pollutants) in conjunction with prolonged exposure to low levels (or otherwise considered to be at or below the national standards) of air pollution. While much of the published work to date has focused on systemic and electrocardiographic consequences of exposure, very few studies have focused on cardiovascular hemodynamics (heart and vascular structure, function, blood flow, etc…). Evidence suggests that it may be possible to detect subtle changes in resting-state cardiac and vascular function in rodents following acute exposure to air pollution using advanced high-frequency ultrasound echocardiography (HF Echo) in vivo. Thus, the primary purpose of the proposed studies is to examine the hemodynamic consequences of exposure to air pollutants, either singly or in combination. Model pollutants to be studied include diesel exhaust particulate matter (DEP), ozone (O3), nitrogen dioxide (NO2), and acrolein, all linked individually to adverse cardiovascular effects.

HF Echo provides a noninvasive means of assessing in vivo cardiovascular function under light anesthesia following an inhalation exposure. A series of HF Echo measurements can be taken across several days following a single or repeated exposure in one animal to identify changes in cardiovascular function across time. Endpoints to be measured include ventricular volumes, cardiac efficiency, heart wall deformation (i.e. wall strain) and changes in blood flow through heart chambers and arteries. Any functional cardiovascular impairment induced by air pollution that is identified in the resting-state with HF Echo should be more profound during cardiovascular challenge and stress. We propose conducting in vivo HF Echo studies in mice before and after air pollution exposure during 1) the resting-state (no cardiovascular challenge) and 2) during pharmacological cardiovascular challenge with an adrenergic activator (e.g. dobutamine) and/or a vasoconstrictor (e.g. thromboxane mimetic). Responses will be examined in two genetic knockout mouse models (B6.FVB(Cg)-Mmp9tm1Tvu/J and B6.129S2-Alox15tm1Fun/J) and a background control (C57BL6/J), all to be obtained from Jackson Laboratories. The B6.FVB(Cg)-Mmp9tm1Tvu/J mice are homozygous null for the matrix metalloproteinase 9 (MMP-9) gene. MMP-9 regulates the integrity of cell to cell junctions in the heart. This is important because each cell to cell junction in the heart must handle extreme changes in load during each cardiac cycle (i.e. the normal pumping of blood). Exposure to air pollution may disrupt how the heart wall withstands changes in load during the cardiac cycle and may be a result of aberrant MMP-9 regulation of cell to cell junction integrity in the heart. The B6.129S2-Alox15tm1Fun/J mice are homozygous mutant/deficient for the arachidonate 15-lipoxygenase (ALOX-15) gene. ALOX15 functions to metabolize arachidonic acid to produce biologically active hydroxy-eicosatetraenoic acids (HETEs). Upregulation of ALOX15 causes macrophage activation and contractile dysfunction in the heart. Moreover, ALOX-15 expression correlates with cardiovascular detriments following traffic pollution exposure in humans.

In addition to HF echo assessments, we propose including separate cohorts of animals to assess cardiac function ex vivo. This technique involves the assessment of excised hearts from which changes in cardiac pace maker function, left ventricular pressure, and coronary vascular resistance can be determined. These studies will enable us to determine if the hemodynamic changes observed in vivo are due to intrinsic changes in heart muscle performance, autonomic function, and/or vascular function.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

Dynamic interplay between the lungs, cardiovascular system, immune system, endocrine organs, and the autonomic nervous system are paramount in understanding how physiological systems respond to environmental stimuli like air pollution. We wish to compare endpoints measured in vivo with endpoints measured ex vivo in isolated organs (heart) in order to identify critical interplay between organ systems that contribute to cardiovascular dysfunction following air pollution exposure. To date, the use of animals is the only means available to study this level of complexity.

b. Justify the species requested:

The mouse is the species of choice for our studies because:

1) The mouse is a mammalian model that allows experimental approaches in situ, in vivo, and ex vivo isolated organs, coupled with the ability to utilize genetic modification (knockouts) to understand how specific genes can

impact organ systems responses to air pollution exposure.

2) Mouse models are well described and utilized frequently in cardiovascular toxicity and pathology studies. This will allow us to examine how our findings relate to other pertinent data in the greater scientific community.

3) Our laboratories, and those of our collaborators, are moving into mouse model studies. We wish to utilize mouse models in this study so that we can broaden our base for future toxicity studies in mouse models.

3. How was it determined that this study is not unnecessary duplication?

Searches of the entire air pollution health research database via Pub-Med, as well as the current Integrated Scientific Assessment document for Particulate Matter, Ozone and Nitrogen Dioxide (using search terms including echocardiography, ex vivo isolated cardiac perfusion, mouse, cardiovascular, MMP9 and ALOX15), have yielded no studies similar to those proposed below; thus, these studies will not duplicate any previously conducted research.

SECTION B - In Vivo Procedures

1. Briefly describe experimental design. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

This project will be divided into 2 studies in male, 8-11 week old C57BL/6J mice (as genetic controls), with two accompanying genetic knockout models on the same background (MMP9-/- and ALOX15 -/-), with average weights 24 - 27 grams. The aims of the studies will be to establish our targeted endpoints (a. heart wall strain in vivo, b. left ventricular filling in vivo, c. pulmonary artery flow in vivo, d. and global coronary artery vascular resistance ex vivo) before and after exposure to single air pollutants alone or in combination. Exposure to air pollution may not result in overt indications of toxicity during assessment of a, b, c, and d during baseline cardiac function. We will then use pharmacological challenge with dobutamine and/or U46619 (thromboxane mimetic) to induce cardiovascular stress, which should make manifest any latent differences in a, b, c, and d following exposure to air pollution.

Study 1 will examine the effects of exposure to the single pollutants diesel exhaust particulate matter (DEP; 500, 150 µg/m3), nitrogen dioxide (NO2; 0.5, 0.2 ppm), Ozone (0.5, 0.2) Acrolein (3, 0.5 ppm) or filtered air (FA). Mice will undergo in vivo HF Echo assessments the day before exposures (time corresponds to time of assessments after exposure) and the day after exposures. A separate cohort of mice will be euthanized for ex vivo isolated perfused heart assessments.

Study 2 examines the effects of co-exposure to two pollutants, i.e., DEP (500 µg/m3) ± NO2 (0.5 ppm) or exposure to DEP (500 µg/m3) ± Ozone (0.5 ppm) or FA. Mice will undergo in vivo HF Echo assessments the day before exposures (time corresponds to time of assessments after exposure) and the day after exposures. A separate cohort of mice will be euthanized for ex vivo isolated perfused heart assessments.

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

We have determined from previous studies that a group size of 6 allows for sufficient power in the statistical analysis of various physiological endpoints. Note: Due to pharmacological challenges, and thus pharmacological desensitization, using separate animals for HF Echo and isolated organs will be critical, thus requiring two cohorts of mice. The following is a description of exposure groups and animal numbers:

Study 1a: Acute concentration-response to DEP

- FA: N = 6 x 3 genetic strains x 2 cohorts (HF echo and ex vivo) = 36
- DEP 500 µg/m3: N = 6 x 3 genetic strains x 2 cohorts = 36
- DEP 150 µg/m3: N = 6 x 3 genetic strains x 2 cohorts = 36

Study 1b: Acute concentration-response to NO2

- FA: N = 6 x 3 genetic strains x 2 cohorts (HF echo and ex vivo) = 36
- NO2 0.5 ppm: N = 6 x 3 genetic strains x 2 cohorts = 36
- NO2 0.2 ppm: N = 6 x 3 genetic strains x 2 cohorts = 36

Study 1c: Acute concentration-response to Ozone (O3)
- FA: N = 6 x 3 genetic strains x 2 cohorts (HF echo and ex vivo) = 36
- O3 0.5 ppm: N = 6 x 3 genetic strains x 2 cohorts = 36
- O3 0.2 ppm: N = 6 x 3 genetic strains x 2 cohorts = 36

Study 1d: Acute concentration-response to Acrolein
- FA: N = 6 x 3 genetic strains x 2 cohorts (HF echo and ex vivo) = 36
- Acrolein 3 ppm: N = 6 x 3 genetic strains x 2 cohorts = 36
- Acrolein 0.5 ppm: N = 6 x 3 genetic strains x 2 cohorts = 36

Study 1 total = 432 mice

Study 2a: DEP+NO2 Acute co-exposures
- FA: N = 6 x 3 genetic strains x 2 cohorts (HF echo and ex vivo) = 36
- DEP 500 µg/m3: N = 6 x 3 genetic strains x 2 cohorts = 36
- NO2 0.5 ppm: N = 6 x 3 genetic strains x 2 cohorts = 36
- DEP 500 µg/m3 + NO2 0.5 ppm: N = 6 x 3 genetic strains x 2 cohorts = 36

Study 2b: DEP+O3 Acute co-exposures
- FA: N = 6 x 3 genetic strains x 2 cohorts = 36
- DEP 500 µg/m3: N = 6 x 3 genetic strains x 2 cohorts = 36
- O3 0.5 ppm: N = 6 x 3 genetic strains x 2 cohorts = 36
- DEP 500 µg/m3 + O3 0.5 ppm: N = 6 x 3 genetic strains x 2 cohorts = 36

Study 2 total = 288

Total mice = 720

Please note that all mice designated for echocardiography assessments are designated under Pain Category C, while those planned for ex vivo cardiac perfusion studie have been designated Category D because of the requirement for removal of heart while the animal is still alive and all those designated for acrolein exposure are under Category E because of the irritant effects of acrolein.

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	306	
D) Potential pain/distress relieved by appropriate measures:	306	
E) Unrelieved pain/distress:	108	

4. For tracking purposes, please check if this LAPR includes any of the following:

☐ Restraint (>15 Minutes) ☐ Survival surgery
☐ Food and/or water restriction (>6 Hours) ☒ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

Whole body inhalation:
To achieve whole body exposures, mice will be placed in nose-only tubes designed for rats and attached to a 52-port flow by nose-only inhalation tower. Animals will be exposed to diesel exhaust particulate matter (DEP) plus/minus Ozone or Nitrogen Dioxide for 3 hours once (acute). Food and water will be restricted during exposure. There will be a minimum of 2 days of acclimation prior to exposure. Chamber temperature, humidity, and air flow will be continuously monitored throughout the exposures. This system is located in Exemption 6 Exemption 6 and the exposure conditions have been previously validated and approved. Particles are delivered to a jet nebulizer by a string feed and blown into the chamber at a constant rate. Particle concentration is determined gravimetrically on

Teflon filters (45 mm diameter with 1 µm pore size; VWR Scientific, West Chester, PA) using a Mercer cascade impactor (Intox Products, Albuquerque, NM), and real-time PM concentration is estimated with an aerosol monitor (Dust Track; TSI Inc., St. Paul, MN) on the chamber exhaust. The concentrations of PM will be similar to those used in previous studies and are not expected to induce weight loss or chronic lung pathology. The maximum PM concentration will not exceed 0.5 mg/m3. Ozone is delivered via a jet nebulizer and blown into the chamber at a constant rate. The ozone generator is a Gas Phase Titration Diluter built for the Environmental Protection Agency by Research Triangle Institute (Research Triangle Park, NC). The maximum Ozone concentration will be 0.5 ppm. NO2 gas will be metered in to the chamber from a stock gas cylinder source. The control system will include appropriate safety valves and gas flow metering devices to allow either manual or automatic injection of NO2 into chamber supply air upstream of the exposure chamber. Chamber test gas concentration will be continuously monitored and recorded by gas analyzers and the chamber data acquisition system (DAS). For automatic exposure control, the DAS will utilize a feedback control loop to regulate injected test agent such that the chamber concentration will be maintained at the target level for the duration of the exposure.

Alternate NO2 and O3 whole body exposure system: In the event that the system using the nose-only tubes is unavailable, we may instead expose mice in whole body chambers made of stainless steel and glass construction allowing visual observation of test animals during exposures. Animals will be housed in wire mesh caging; this system is located in room Exemption 6 Exemption 6. The generation of O3 and NO will be the same as described above.

b. Survival Blood Collections (method, volume, frequency):

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):

Ultrasound Echocardiography: Cardiac function will be determined via HF Echo using a VisualSonics Vevo 2100 ultrasound system in room Exemption 6 Exemption 6 Exemption 6. Mice will be placed in a sealed chamber and anesthetized with 2-5% Isoflurane delivered in 100% O2 at 0.8-1 L/min. We will then move mice to a heated procedure table where anesthesia will be maintained with 1-4% Isoflurane (100% O2 @ 0.8-1 L/min) via a nose cone. The eyes will be coated with eye lubricant to prevent drying of the eyes during the procedure. Each paw will be gently taped to ECG electrodes coated with electrode gel (to monitor heart rate and respiratory rate) and body temperature will be monitored with a rectal probe. Nair gel will be used to remove fur from the imaging location (chest and upper abdomen). The application area will be washed to remove any residual Nair from the skin. Prewarmed ultrasound gel will be applied to the chest and the HF Echo transducer will be used to noninvasively record 3 video loops of a) blood flow into the left ventricle; b) blood flow through the pulmonary artery; and c) the contractile motion of the heart. Each of these measurements will be made one day prior to exposure and one day after exposure. After the measurements are made on the day prior to exposure, mice will be gently and carefully cleaned of all transducer gel. Mice will then be removed from anesthesia and allowed to recover in fresh clean cages. Mice will be monitored during the recovery period until normal grooming habits resume before returning to animal holding room Exemption 6 Exemption 6. Note: Precautions will be taken to ensure that freshly clean cages do not come into contact with any lab surface. On the day after exposure, after the baseline measurements (a, b, and c) have been collected the transducer will be maintained in a position to measure blood flow into the left ventricle (a). A 27 G needle will be used to infuse a 0.1 mL bolus of 5 µg/kg dobutamine (alpha-adrenergic agonist) into the lateral tail vein and blood flow into the left ventricle (a) will be recorded at 2 minutes and 5 minutes post-infusion. Mice will be given approximately 15 minutes to return to baseline heart rate, during which time the transducer will be positioned to measure pulmonary artery flow (b). A 27 G needle will then be used to infuse a 0.1 mL bolus of 0.3 µg/kg U46619 (thromboxane mimetic) into the lateral tail vein and pulmonary artery flow will be measured 2 minutes after infusion and 5 minutes after infusion. Then a 0.1 mL bolus of 1 µg/kg of U46619 (dose increased by a half log) will be infused into the lateral tail vein and changes in pulmonary artery flow will assessed 2 minutes and 5 minutes after infusion. Mice will then be euthanized for necropsy. Notes: 1) Anesthesia time should be a short as possible but during the pharmacological challenges anesthesia time will likely last longer than 30 minutes. In this case body temperature will be noted throughout the procedure at 5-7 minute intervals. 2) Pharmacological challenges during HF Echo assessments will only take place after exposure. Pre-exposure echo assessments will not involve drug challenge.

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):

f. Describe how animals will be monitored (e.g., frequency of observations, by whom):
Whole body exposures: Mice will be visually monitored at least once every hour during exposure and up to two hours after exposure by Exemption 6Exemption 6Exemption 6Exemption 6Exemption 6 .

Ultrasound echocardiography: The Vevo 2100 Ultrasound system comes equipped with an electrocardiogram (ECG) platform on which the anesthetized animal will be placed and monitored. This will enable measurement of ECG, heart rate, and respiratory rate by the individual performing the assessments (i.e., Exemption 6 Exemption 6Exemption 6Exemption 6 The system also includes a rectal probe to measure internal body temperature. All values will be prominently displayed on the screen enabling monitoring from start to finish. After removing the mouse from the anesthesia induction chamber, eye ointment and Nair are applied and each limb paw will be placed on an electrode contact to monitor ECG, heart and respiratory rates. The rectal probe will then be inserted to monitor body temperature. All vital signs will be monitored until the animal is returned to its home cage in the case of pre-exposure assessments or in the instance of euthanasia, administration of sodium pentobarbital.

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):
Acrolein exposure
Groups of mice will be exposed to a single concentration of acrolein (3.0 or 0.5 ppm) for 3 hours via whole-body inhalation in room Exemption 6 Exemption 6 Exemption 6. Acrolein gas will be metered from a 1000ppm cylinder into a glass mixing chamber where the gas will be mixed with dry filtered dilution air to achieve the final concentration of acrolein with a total flow of 6L/min. All animals will be monitored in a whole-body plethysmograph (WBP - Model PLY3213, Buxco Electronics, Inc, Wilmington, NC). Petrolatum (Puralube Vet) ointment will be placed on the eyes before exposure to ameliorate or prevent ocular irritation. Following exposure, animals will be returned to their home cages.

b. Survival Blood Collection (method, volume, frequency):
na

c. Testing methods:
na

d. Restrictions placed on the animals' basic needs (e.g., food and/or water deprivation, light cycles). Provide details regarding the length of deprivation:
na

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):
Mice will be visually monitored at least once every hour during exposure and up to two hours after exposure by Exemption 6Exemption 6Exemption 6Exemption 6Exemption 6 .

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:
na

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:
na

7. Surgical Category D and E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:
Excision of Heart for Isolated Perfusion Technique
Mice will be administered a single injection of 200 mg/kg sodium pentobarbital IP within 24 hours after exposure to air pollution or filtered air. This is delivered diluted by saline (total volume ~ 400 microliters) with a 25 G needle (one needle per mouse) is used. Mice are tested for surgical plane of anesthesia by multiple firm toe pinches to hind feet. If no response is seen, the animal is clipped, wiped with alcohol, and the incision is made with clean scissors and forceps. The abdomen is then opened and the inferior vena cava injected with 0.1 ml (100 units) of heparin to prevent clotting in order to prepare for the isolated heart reperfusion technique. The heart is then completely removed and suspended in buffer to perform the isolated preparation. Mice are carefully monitored during the entire process.

b. Anesthetic regimen (drugs, dosages, volume, and route of administration). The use of paralytic or neuromuscular blocking agents without anesthesia is prohibited:

A single IP injection of 200 mg/kg sodium pentobarbital

c. Postoperative care (thermal support, special feeding, frequency and duration of monitoring, responsible personnel, removal of sutures/staples):

na

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

na

e. Will any animals be subject to more than one major surgical survival procedures?

○ Yes ● No

f. Identify any surgical procedures performed at other institutions or by vendors:

na

8. Humane interventions (for treatments/procedures in all categories).

a. Describe actions to be taken in the event of expected or unexpected deleterious effects from procedures or chemical exposures.

All animals will be monitored visually (obvious distress, gait, breathing, appetite, etc.) at least twice daily. Animal weight will be tracked routinely in case of sudden weight loss (>10%). Mice not weighed routinely will be examined for body condition to determine health status. If signs of distress or other deleterious effects are observed, all animals from the treatment group will be isolated in a clean control atmosphere and observed for recovery trends. They may be reused for the study if recovery is demonstrated; otherwise, they will be euthanized. The attending veterinarian may be consulted to determine the appropriate course of action.

b. State criteria for determining temporary or permanent removal of animals from the study.

One or more of the following: weight loss (> 10%), labored breathing, abnormal gait, and loss of appetite.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

Assessment of isolated perfused hearts is the only reliable method for directly assessing intrinsic functional deficits in the intact heart in the absence of other factors (changes in blood pressure and autonomic innervation) as determined by searches in Pubmed on April 14, 2014.

Acrolein exposures have been conducted previously by us and others at EPA. Ocular irritation is ameliorated with lubricant, however, to fully understand the irritant effects of this gaseous pollutant, animals need to be exposed (whole-body) without any respiratory interventions to determine the cardiopulmonary physiological (irritant) responses.

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study:	720
b. Animals to be transferred from another LAPR:	
LAPR Number that is the source of this transfer:	
c. Animals to be transferred from another source:	
d. Offspring produced onsite (used for data collection and/or weaned):	
e. TOTAL NUMBER of animals for duration of the	720

LAPR

2. Species (limited to one per LAPR): Mouse/Mice

3. Strain: C57BL/6J (wildtype);
B6.FVB(Cg)-Mmp9tm1Tvu/J
and B6.129S2-Alox15tm1Fun/J)

Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

na

4. Sources of animals:

Jackson Laboratories

5. Provide room numbers where various procedures will be performed on animals:

Exemption 6

- Initial Housing

Exemption 6: Whole-body exposure to air pollutants

Exemption 6 Acrolein exposures

Exemption 6 Ultrasound echocardiography and necropsy

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

na ***Room Numbers:***

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

na

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

na

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

na

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

Three to five mice will be housed in each solid bottom cage with alpha dry bedding. Envirodry and nestlets will be provided for enrichment.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.
Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for

dosing.

Air Pollutants:
Diesel Exhaust Particulate Matter: No LD50 known or available. Maximum dose (based on PM concentration) = 500 micrograms/m3 for inhalation studies

Ozone: Inhalation LC50 for 3 hour exposure in mice = 12.6 ppm. Maximum dose = 0.5 ppm

Nitrogen dioxide - Inhalation LC50 for 0.67hours = 1000ppm; maximum dose = 0.5 ppm. The HSRP for Nitrogen dioxide --- HSRP 778 entitled "Small Animal Inhalation Exposure to NO2

Acrolein (2-propenal, acraldehyde, allyl aldehyde, acryl aldehyde):
HSRP ID: 79; Title: Inhalation Exposures to Various Vapors and Gases; Approved: 3/9/95
The reported LC50 values for acrolein vapor are:
66 ppm (6h), mice, combined sexes (Ballantyne et al., Hum. Toxicol. 8:229–35, 1989)
8 ppm (4h), mice (Carpenter et al., J. Ind. Hyg. Toxicol. 31:343-346, 1949).

Drugs:
Dobutamine hydrochloride (Pharmaceutical grade): maximum dose 10 micrograms/kg; Mouse iv LD50 = 34 milligrams/kg

U46619 (thromboxane mimetic)- Maximum dose 1 ug/kg LD50 Intravenous: 66 ug/kg

Heparin: (Pharmaceutical grade; 100 units per mouse in 0.1ml). Mouse LD-50 is 391211 IU /kg.

Saline (Pharmaceutical grade): a non-hazardous agent used as a vehicle for drugs used for cardiovascular challenge; maximum dose = 500 microliters.

Anesthetics
Sodium pentobarbital/phenytoin: Maximum dose of Na pentobarbital to be administered = 200 mg/kg; Maximum dose of Phenytoin to be administered = 25 mg/kg). Pentobarbital LD50 mouse, oral = 239 mg/kg. Phenytoin LD50 mouse, oral = 150 mg/kg. We will consider using a pentobarbital stock without phenytoin.

Isoflurane: Maximum concentration is 3%. LC50 mouse, inhalation = 16,800 ppm. Isoflurane is considered a "potentially hazardous substance" but does not require an HSRP. Isoflurane will be used in the chemical safety hood in A-579 or A-587, and standard PPE (safety glasses, gloves, lab coat) will be worn by all personnel at all times while being used.

2. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in this LAPR, and provide:

a. Information to assure that such material is pathogen-free
na

b. A statement regarding any safety precautions necessary for handling the material.
na

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform

the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	study coordination; animal handling/care; surgical procedures; physiological monitoring	>15 years of experience in use of laboratory animals; > 7 years surgical procedures and physiological monitoring; completed NHEERL animal use/care training
Exemption 6	Post-Doc	study coordination; animal handling/care; surgical procedures; physiological monitoring	4.5 years of experience in use of laboratory animals; 2.5 years, surgical procedures; completed NHEERL animal use/care training
Exemption 6	Associate Principal Investigator	study coordination; animal handling/care; surgical procedures; physiological monitoring	>15 years of experience in use of laboratory animals; > 7 years surgical procedures and physiological monitoring; completed NHEERL animal use/care training
Exemption 6	Technical Staff	animal handling/care; surgical procedures; physiological monitoring	>20 years of experience in use of laboratory animals; 9 years, surgical procedures; completed NHEERL animal use/care training
Exemption 6	Technical Staff	animal handling/care; surgical procedures; physiological monitoring	>20 years of experience in use of laboratory animals; 9 years, surgical procedures; completed NHEERL animal use/care training
Exemption 6	Technical Staff	animal handling/care; whole-body inhalation procedures	>20 years of experience in use of laboratory animals and inhalation exposures; completed NHEERL animal use/care trainin
Exemption 6	Student	animal handling/care; whole-body inhalation procedures	~1 year of experience in use of laboratory animals, and physiological monitoring; completed mouse handling 101 and NHEERL animal use/care training
Exemption 6	Student	animal handling/care; whole-body inhalation procedures	>3 years of experience in use of laboratory animals, and physiological monitoring; completed mouse handling 101 and NHEERL animal use/care training
Exemption 6 Exemption 6 Exemption 6	Student	animal handling/care; whole-body inhalation procedures	>3 years of experience in use of laboratory animals, and physiological monitoring; completed mouse handling 101 and NHEERL animal use/care training
RTP-NHEERL	Tech Support	Category C Procedures	EPA IACUC Trained

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year	na
2. Breeding protocols and recordkeeping	na
3. Methods for monitoring genetic stability	na
4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR	na

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

No later than 24 hours after exposure to air pollution

2. Describe the euthanasia techniques:

Method(s):	Anesthesia plus vital organ transsection
Agent(s):	Pentobarbital
Dose (mg/kg):	200 mg/kg
Volume:	~0.4 mL
Route:	intraperitoneal

Source(s) of information used to select the above agents/methods:

Personal Experience, 2007 AVMA Guidelines on Euthanasia.

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the 2007 American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).

4. Describe how death is to be confirmed.

Vital organ section

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.

Transferred to another study

The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?

● Yes ○ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.

2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.

3. The proposed research using animals does not unnecessarilty duplicate any previous experimentation.
4. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
5. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. Individuals from outside of EPA who are collaborating on this project, and who conduct related experimentation on EPA procured or bred animals in their respective Institutions, have the equivalent of a current IACUC approved LAPR at their respective Institutions.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 by Exemption 6	04/14/2014

Submitted: 04/15/2014

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	*Approval Date*	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	04/15/2014 by Exemption 6 Exemption 6/RTP/USEPA/US	Exemption 6 *Lotus Notes Address* Exemption 6 Exemption 6/RTP/USEPA/US	EPHD *Branch* CIB	MD *Submitted to Branch Chief for Approval* 04/15/2014 09:55 AM

ATTACHMENTS

17-05-001 resp.pdf Exemption 6 IRP___IRP-NHEERL-RTP-EPHD-CIB-14-01-001.pdf

Actions

First Update notification sent: 03/27/2015
Second Update notification sent:
First 2nd Annual notification sent: 04/13/2016
Second 2nd Annual notification sent:

1st Expiration notification sent: 04/03/2017
2nd Expiration notification sent: 05/02/2017

History Log: